

CD133 expression in chemo-resistant Ewing sarcoma cells.

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Public Summary:

BACKGROUND: Some human cancers demonstrate cellular hierarchies in which tumor-initiating cancer stem cells generate progeny cells with reduced tumorigenic potential. This cancer stem cell population is proposed to be a source of therapy-resistant and recurrent disease. Ewing sarcoma family tumors (ESFT) are highly aggressive cancers in which drug-resistant, relapsed disease remains a significant clinical problem. Recently, the cell surface protein CD133 was identified as a putative marker of tumor-initiating cells in ESFT. We evaluated ESFT tumors and cell lines to determine if high levels of CD133 are associated with drug resistance. **METHODS:** Expression of the CD133-encoding PROM1 gene was determined by RT-PCR in ESFT tumors and cell lines. CD133 protein expression was assessed by western blot, FACS and/or immunostaining. Cell lines were FACS-sorted into CD133+ and CD133- fractions and proliferation, colony formation in soft agar, and in vivo tumorigenicity compared. Chemosensitivity was measured using MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assays. **RESULTS:** PROM1 expression was either absent or extremely low in most tumors. However, PROM1 was highly over-expressed in 4 of 48 cases. Two of the 4 patients with PROM1 over-expressing tumors rapidly succumbed to primary drug-resistant disease and two are long-term, event-free survivors. The expression of PROM1 in ESFT cell lines was similarly heterogeneous. The frequency of CD133+ cells ranged from 2-99% and, with one exception, no differences in the chemoresistance or tumorigenicity of CD133+ and CD133- cell fractions were detected. Importantly, however, the STA-ET-8.2 cell line was found to retain a cellular hierarchy in which relatively chemo-resistant, tumorigenic CD133+ cells gave rise to relatively chemo-sensitive, less tumorigenic, CD133- progeny. **CONCLUSIONS:** Up to 10% of ESFT express high levels of PROM1. In some tumors and cell lines the CD133+ fraction is relatively more drug-resistant, while in others there is no apparent difference between CD133+ and CD133- cells. These studies reveal heterogeneity in PROM1/CD133 expression in ESFT tumors and cell lines and confirm that high levels of PROM1 expression are, in at least some cases, associated with chemo-resistant disease. Further studies are required to elucidate the contribution of PROM1/CD133 expressing cells to therapeutic resistance in a large, prospective cohort of primary ESFT.

Scientific Abstract:

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difference between CD133⁺ and CD133⁻ cells. These studies reveal heterogeneity in PROM1/CD133 expression in ESFT tumors and cell lines and confirm that high levels of PROM1 expression are, in at least some cases, associated with chemo-resistant disease. Further studies are required to elucidate the contribution of PROM1/CD133 expressing cells to therapeutic resistance in a large, prospective cohort of primary ESFT.

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